機能性オリゴヌクレオチドの開発と遺伝子発現制御への展開

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博士論文

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Contents

Oligonucleotides (ONs) are useful chemical tools and applied for manipulating gene expression and detecting a specific DNA and RNA sequence. So far, various types of chemically modified ONs have been developed to improve the property of ONs. However, the development of more high-performance ONs is still demanded. In this thesis, we aimed to construct novel strategy to regulate gene expression, and developed novel functional oligonucleotide.

To expand the target base of (AOVP), we newly designed AOVP derivative (**2**) which possessed short and flexible linker unit, and demonstrated that the novel AOVP derivative reacted with guanosine residue in complimentary DNA and RNA with moderate reactivity. Furthermore, we have applied the crosslink reaction of **2** to guanosine derivatives including inosine and 8-oxoguanosinee based on the crosslink reactivity of **2** to guanosine, and clarified that **2** reacts with 8-oxoguanosine (8-oxoG) in high yield and selectivity by utilizing the difference of p*K*a (N1) of guanosine derivatives. To our best knowledge, this is first example of the selective crosslink reaction to 8-oxoguanosine. We expected that **2** would be enabled to apply the sequence selective detection of 8-oxoG (Chapter 2)

The approach to give an external stimulation responsibility for molecules is very effective to control the functions of molecules at intended place and timing. We have developed a novel oxidation triggered crosslink nucleobase ATVP (**1**) and demonstrated that the oxidized form ASVP (**2**) showed very fast and selective crosslink reaction to cytosine in RNA. Furthermore, **1** in duplex was oxidized by H_2O_2 and $FeCl_2$ thereby indicating crosslink reactivity. We are considering that these results strongly suggest the capability of **1** for regulating gene expression in cell by optimizing oxidation potential of sulfide moiety of **1** (Chapter 3).

The specific interaction between biomolecules and metal ions is crucial event to induce their function. Inspired by the behavior of metal ions in nature, we have developed the metal ion triggered crosslink reaction using ATVP and demonstrated that $NiCl_2$ specifically activated the crosslink reactivity. We consider that this result provided valuable information to design a novel external stimuli responsible crosslink forming molecule (Chapter 4).

We have synthesized the 6-AVP derivatives, expected to crosslink with thymine, and evaluated the crosslink reactivity. The ON containing the non-substituted 6-AVP

derivative (**2a**) can form a stable duplex with the target DNA under the stated reaction conditions but did not produce any crosslink formation toward the target DNA or RNA, due to the low electrophilicity of the vinyl group. On the other hand, the introduction of a methyl ester group onto the vinyl group of the 6-AVP derivative (**2b**) increased the crosslink reactivity to produce the adducts except for the thymine target base. These results have provided useful information for the design of new crosslink forming agents (Chapter 5).

Many human genetic diseases are caused by nonsense mutations that lead to premature stop codon (PTC) in open-reading frame on mRNA and eventually to truncated and nonfunctional proteins. Aminoglycoside (AG) antibiotics can make ribosome read-through PTCs and produce full-length protein, though there are some limitations for therapeutic use of AGs due to high toxic (nephrotoxity and ototoxity) and insufficient read-through activity at subtoxic doses. In order to address the problematic issues of an existing read-through methodology, we have developed a novel read-through methodology using ONs. We have clarified that ON can allows ribosome to read-through, and that the coexistence of ON and AG significantly improve the read-through efficiency with synergic effect (Chapter 6).